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# The influence of reduced tumor suppressor protein 53 (p53) on the activity of Phosphatase of Regenerating Liver-1 (Prl-1) in Drosophila melanogaster

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# The influence of reduced tumor suppressor protein 53 (p53) on the activity of Phosphatase of Regenerating Liver-1 (Prl-1) in *Drosophila melanogaster*

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## Background:

PRL-1 is one member of a family of phosphatases that have been reported to act in promoting cancer. In particular, over-expressed human PRL-3 plays a significant role in contributing to metastatic migration of a myriad of cancers such as colorectal, liver, and prostate cancers (Min *et. al.*, 2009). However, previous work shows that in healthy, non-cancerous cells, PRLs instead aid in the prevention of uncontrolled cellular division (Basak *et. al.*, 2009). This suggests that PRLs can function in opposing ways that likely reflect the particular genetic environment of the cell. One focus of our lab is to identify genetic changes common to cancerous cells that enable PRLs to contribute to cancer.

My work focuses on the tumor suppressor p53, which is found to be mutated in approximately 50% of human cancers (Rodrigues *et al.*, 1990). In doing so, addressing the relationship between PRL-1 and p53 in *Drosophila*, a model organism that allows for easy genetic manipulation. Within this model I am focusing on intestinal stem cells (ISCs). ISCs are particularly useful in that they share several hallmarks with cancer cells, they are undifferentiated and possess an unlimited capacity for growth. These ISC also form small clusters of relatively small cells (~3-4) that make them easy to distinguish from neighboring cells (Figure 1).

By overexpressing PRL-1 with reduced p53 gene expression, I can determine whether PRL-1 either contributes to or counteracts the ability of the ISCs to demonstrate other properties of cancer cells, such as increased cell division or migration. My previous work showed that reducing p53 with overexpressing PRL-1 leads to increased numbers of ISCs and less discrete, indistinguishable cluster morphology (Figure 3).

The goals of this project are to directly test whether these extra cells are due to cell division or migration and to quantify whether the alterations in ISCs affects viability of the adult organism (Figure 2).

## Methods:

The gene PRL-1 was overexpressed using an Upstream Activating Sequence (UAS) in combination with escGAL4, a protein that promotes gene expression specifically in intestinal stem cells. P53 was reduced using the UAS/GAL4 system as in conjunction with RNA interference (RNAi), which binds to p53 RNA, marking it for destruction (preventing protein function).

Three crosses were performed to acquire offspring with genotypic traits of interest. In order to detect ISCs that are undergoing cell division, Bromodeoxythymidine (BrdU) was utilized during larval stages. BrdU is a synthetic analogue for the DNA base nucleotide Thymidine. When incubated with BrdU, cells uptake this nucleotide substitute into newly replicating DNA. Using antibodies to BrdU, these cells were then detected with a fluorescent marker, under UV light (Figure 2, 4). In order to assess viability of particular gene expression, the genotypes of adult offspring were quantified and compared to expected frequencies (Figure 5).

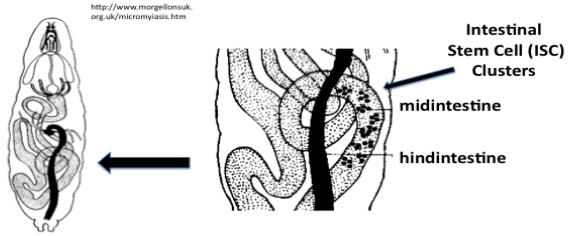


**Figure 1. Schematic of *D. melanogaster* larva intestine.** Intestinal Stem cells clusters tend to be found particularly in the midintestine.

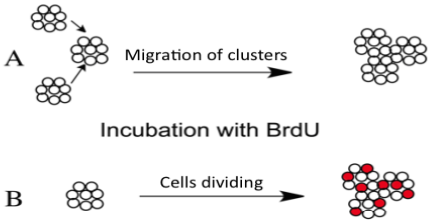


**Figure 2. Scheme depicting two possibilities for ISC growth.** Increased numbers of ISCs per cluster could result from either A) migration or B) division. Incorporation of BrdU will distinguish between the two.

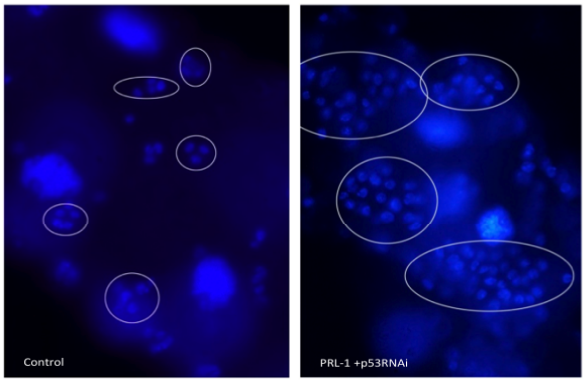


**Figure 3. Intestines from *Drosophila* larvae.** ISC clusters are circled. The Control image shows stem cells with PRL-1 and p53 at normal levels. Much larger ISC clusters are seen in intestines from animals with increased PRL-1 and reduced p53 expression.

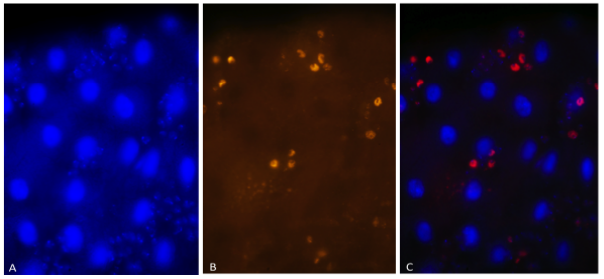


**Figure 4. Detection of ISC undergoing DNA replication.** Intestinal stem cells stained for either DNA in blue or BrdU in red. A) Staining for DNA alone. B) Staining for BrdU alone. C) Combined BrdU and DNA staining.

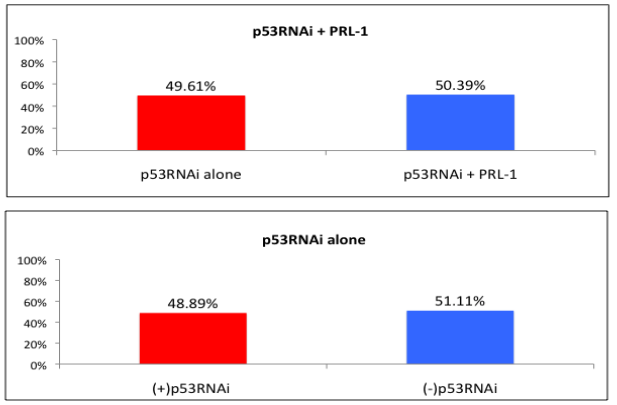


**Figure 5. Examining whether reduced p53 with or without increased PRL-1 results in lethality.** Crosses were performed with female *Drosophila* carrying esgGal4 and males carrying genes to control expression of either p53 or PRL-1. The genetically different offspring were then quantified and numbers compared to expected ratios to determine if a genotype was lethal. No evidence of lethality was discovered.

## Summary:

While BrdU staining was successful, there were inconsistencies in DNA staining so ISCs that did not incorporate BrdU were unable to be identified. This prevented quantification of the percentage of ISCs undergoing DNA replication. However, absolute BrdU staining between genotypes was measured and showed no changes in the amount of cellular division taking place. Overall suggesting migration as the primary mechanism for the increased size of ISC clusters.

Future work could involve reworking the assay to gain better DNA staining of the stem cells not undergoing BrdU uptake.

Analysis of survival rates for *Drosophila* expressing either p53RNAi or p53RNAi + PRL-1 showed no evidence of lethality. This indicates that large increase of ISCs have little effect on the ability of the fruit fly to survive to adulthood.

## Literature Cited:

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